BioDiscovery 20: e11207
doi: 10.3897/biodiscovery.20.e11207

OPEN ACCESS

Research Article

Phosphorylase Kinase Inhibition Therapy in Burns and Scalds

Madalene Heng ‡

‡ UCLA School of Medicine, Los Angeles, United States of America

Corresponding author: Madalene Heng (madalene@madalenehengmd.com)

Academic editor: Nikolai Zhelev

Received: 15 Nov 2016 | Accepted: 02 Feb 2017 | Published: 24 Feb 2017

Citation: Heng M (2017) Phosphorylase Kinase Inhibition Therapy in Burns and Scalds. BioDiscovery 20: e11207. https://doi.org/10.3897/biodiscovery.20.e11207

Abstract

Severe burns and scalds almost always result in unsightly hypertrophic scarring. Among the important processes involved in scarring are fibroblast formation and transformation of fibroblasts into myofibroblasts. Myofibroblasts contain α-smooth muscle actin which has contractile properties and can lead to wound contraction and hypertrophic scarring. Phosphorylase kinase (PhK), expressed within 5 mins of injury, is among the earliest enzymes released after tissue damage. It is responsible for activation of NF-kB, which in turn activates over 200 different genes related to inflammation, fibroblastic proliferation, myofibroblast conversion, and eventual scar tissue formation. The sequence and approximate timing of events following injury include the following: activation of PhK (5 mins), followed by appearance of neutrophils (30 mins), macrophages (hours to days), fibroblasts (1 week) and myofibroblasts (2 weeks). Cytokines and growth factors secreted by macrophages include fibroblast growth factor (FGF) and transforming growth factors α and β (TGFα and TGFβ). Fibroblast growth factor is responsible for fibroblastic proliferation, and TGFβ1 for conversion of fibroblasts into myofibroblasts. After thermal injury, the use of topical curcumin, a non-competitive, selective PhK inhibitor that blocks PhK activity upstream of NF-kB activation, was found to be associated with more rapid and improved skin healing, as well as less severe or absent scarring.

Keywords

tissue injury, thermal injury, hypertrophic scarring, fibroblast proliferation, myofibroblast conversion

Mechanisms of Scarring after Thermal Injury

Wound healing following significant burns and scalds almost always result in hypertrophic scarring. In general, all tissues after injury are acutely infiltrated by many cell types (Leibovich and Ross 1975, Springer 1994, Desmouliere and Gabbiani 1996, Martin 1997), including platelets, inflammatory cells (neutrophils, macrophages and lymphocytes), endothelial cells, fibroblasts and epidermal cells. Wound healing involves a series of complex biologic processes, including inflammatory cell migration, cell proliferation, matrix synthesis, and wound contraction. Cytokines and growth factors secreted by inflammatory cells amplify NF-kB-dependent signaling pathways, resulting in fibroblastic proliferation and increased collagen synthesis. Single strands of collagen fibers polymerize and cross-link to form thick strands of collagen fibers. A proportion of fibroblasts transform into myofibroblasts, which possess strong contractile forces and are mainly responsible for wound contraction and hypertrophic scaring (Montesano and Orci 1988, Clark et al. 1989, Desmouliere et al. 1993, Grinnell 1994, Lee et al. 1999, Abdou et al. 2011). During wound healing, certain factors (Heng 2011), including wound location, wound depth (Dunkin et al. 2007, Wang et al. 2008), infection and genetic predisposition, enhance the frequency of hypertrophic scarring. However, thermal injuries secondary to burns and scalds are particularly prone to produce hypertrophic scarring in the skin (Tredget et al. 2006). The understanding of the sequence of events which lead to fibroblast proliferation and myofibroblast conversion, as well as identification of signaling targets on which to focus therapeutic interventions, may lead to more effective prevention of scarring after thermal injuries.

Sequence of Events in Wound Healing Following Injury

We found that one of the earliest cells activated by tissue injury is the Langerhans cell, the activation of which is detected within 5 mins of epidermal injury (Heng and Heng 2014). This is followed by infiltration of the wound by neutrophils as early as 30 mins after injury. Platelets, activated to secure hemostasis, release platelet derived growth factor (PDGF), which is chemotactic to neutrophils. Nuclear factor- kB (NF-kB), which is detected in injured tissue as early as 30 to 60 mins after injury, is expressed by both activated Langerhans cells and neutrophils. The neutrophils clear debris and bacteria, and secrete adhesion molecules (p-selectin) which assists in the migration of neutrophils into wounds. Macrophages and T lymphocytes, which are observed in wounds within hours to days, form the next wave of inflammatory cells. They secrete cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNFα), and growth factors such as FGF and transforming

growth factors (α and β) which stimulate and amplify fibroblastic proliferation (Martin 1997, Heng 2011). Activated fibroblasts are observed one week following injury, and myofibroblasts usually make their appearance 2 weeks following heat injury.

Role of Fibroblasts and Myofibroblasts in Hypertrophic Scarring

Activated fibroblasts infiltrate the wound about a week after injury. TGF-β, which is chemotactic to activated fibroblasts, also stimulates fibroblastic proliferation (Montesano and Orci 1988, Desmouliere et al. 1993, Grinnell 1994, Martin 1997, Heng 2011). When activated, single strands of collagen fibers polymerize and cross-link to form thick strands of collagen fibers, embedded in a newly synthesized metalloprotein-rich extracellular matrix. Burned patients with hypertrophic scarring have been observed to have polarized IL-4+ Th2 cytokine production, with significantly increased IL-10 and TGF-β production (Tredget et al. 2006). TGF-β is a pleiotropic growth factor, secreted by many activated cells, including inflammatory cells such as macrophages. In the process of hypertrophic scarring, inflammatory cells release cytokines such as transforming growth factor-β1 (TGFβ1), which induces the transformation of fibroblasts into myofibroblasts (Montesano and Orci 1988, Desmouliere et al. 1993, Grinnell 1994, Tredget et al. 2006, Heng 2011).

The transformation of fibroblasts into myofibroblasts, which usually occurs 2 weeks after injury, is probably the key event in the formation of hypertrophic scarring. Myofibroblasts express α-smooth muscle actin and possess contractile properties resembling smooth muscle, resulting in generation of the forces that lead to wound contraction and hypertrophic scarring (Montesano and Orci 1988, Desmouliere et al. 1993, Grinnell 1994). The transformation of fibroblasts into myofibroblasts is stimulated by TGFβ1, which is found in high levels in burns and scalds (Heng 2011, Tredget et al. 2006). The formation of myofibroblasts is also enhanced by wound tension, infection, deep wounds (Dunkin et al. 2007, Wang et al. 2008) and an inherited keloidal tendency (Lee et al. 1999, Abdou et al. 2011, Heng 2011). It is noteworthy that hypertrophic scarring is usually not observed in embryos and fetuses (Adzick et al. 1985, Martin 1997, Ferguson and O'Kane 2004), in whom TGFβ1 is only expressed transiently and at low levels (Martin et al. 1993, Martin 1997, Whitby and Ferguson 1991) with resulting absence of myofibroblast conversion (Estes et al. 1994, Armstrong and Ferguson 1995). In contrast, hypertrophic scarring is more commonly observed with adult wounds, where TGFβ1 levels are higher and more prolonged (Montesano and Orci 1988, Desmouliere et al. 1993, Grinnell 1994, Martin 1997, Heng 2011).

Signaling Pathways Induced by Injury

The molecule induced by injury and central to the wound healing process appears to be the transcription activator, NF-kB, which is detected as early as 30 mins after injury (Bethea et al. 1998). In the non-activated state, NF-kB exists as a pair of dimers (p50/p65) present in the cytoplasm. When NF-kB is activated by injury, phosphorylation occurs at Ser-276,

Ser-529 and Ser-536, and the inhibitory molecule (IkBα) is removed so that the p50/p65 dimers can translocate to the nucleus, where it binds to the kB site on the DNA molecule (Verma et al. 1995, Takada et al. 2004, Yang et al. 2004). This is necessary for NF-kB to activate the transcription of over 200 genes involved in cell cycling, cell proliferation, cell migration and anti-apoptosis. The removal of the inhibitory molecule, IkBα, is dependent on activation of its kinase, IkBα kinase. The activation of IkBα kinase requires phosphorylation of multiple sites on the β subunits (Ser 171, Ser 181, Tyr 188. Tyr 199), as well as phosphorylation of the Zn finger on the γ subunit (Yang et al. 2004), which also contains an ubiquitin ligase site. The resultant degradation by ubiquitin–dependent proteolysis frees the NF-kB subunits (Karin and Ben-Neriah 2000, Yang et al. 2004, Palkowitsch et al. 2008), enabling migration of the subunits to their DNA binding site. The synchronization of phosphorylation events in the above phosphorylation sites required for the activation of both NF-kB and IkBα kinase is facilitated by the dual-specificity enzyme, PhK (Yuan et al. 1993, Reddy and Aggarwal 1994, Singh and Aggarwal 1995, Heng et al. 2000, Heng 2010), and inhibited by its inhibitor, curcumin (Reddy and Aggarwal 1994, Singh and Aggarwal 1995, Heng 2010, Heng 2013), (Fig. 1).

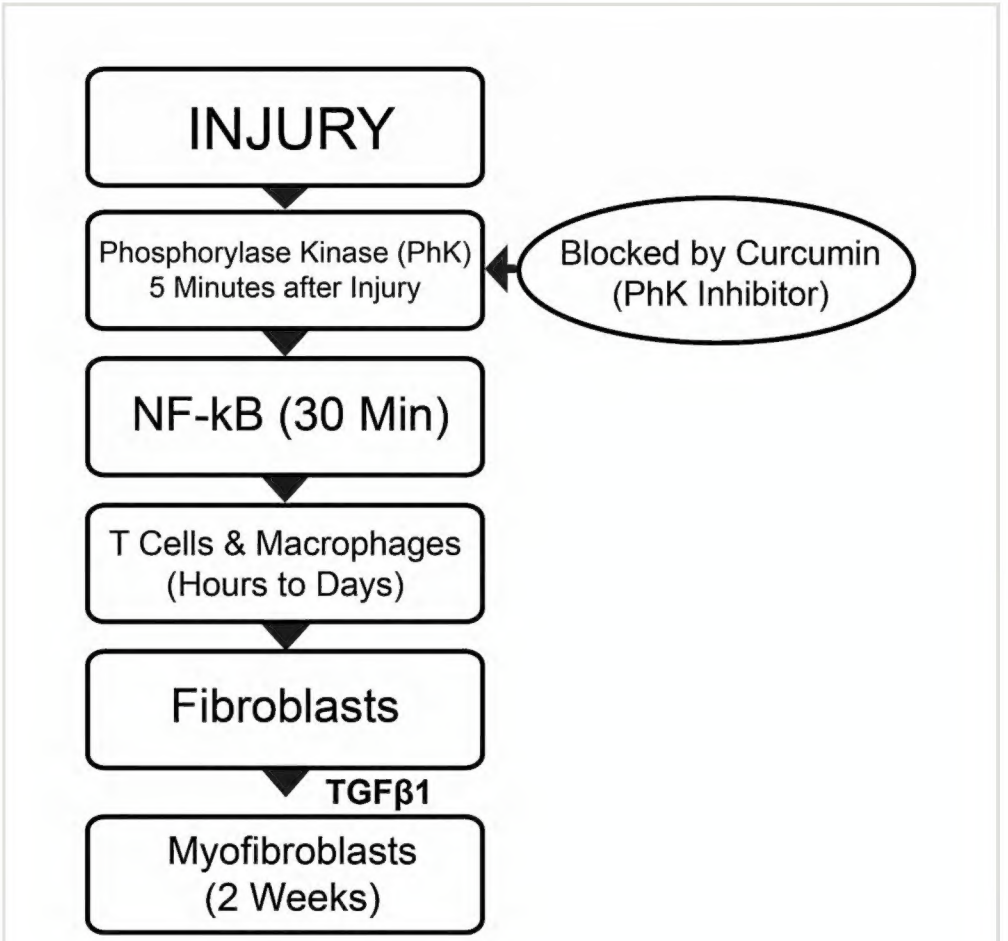

Figure 1.

Signaling pathways and Sequence of Events induced by injury. Approximate time sequences after injury are given within brackets. Abbreviations: NF-kB (nuclear factor-kB), TGFβ1 (transforming growth factor-β1).

Curcumin (PhK Inhibitor) use after Thermal Injury

Phosphorylase kinase is a dual specificity kinase capable of transferring high energy phosphate bonds to both serine/threonine and tyrosine specific substrates (28). Most protein kinases can only transfer high energy phosphate bonds to substrates of a single specificity i.e. either serine/threonine or tyrosine. Phosphorylase kinase is a unique enzyme in which the spatial arrangements of the specificity determinants can be manipulated so that PhK can transfer high energy phosphate bonds from ATP to substrates of different specificities, such as serine/threonine and tyrosine residues (Yuan et al. 1993, Heng et al. 2000). It achieves its unique ability to act as a dual-specificity protein kinase (Yuan et al. 1993) by means of a hinge joint between its subunits, which permits changes in size of the substrate binding site, and also through binding to ions such as Mg or Mn, which allows the shape of the substrate binding site to be altered in different planes.

Curcumin, the active ingredient of the spice, turmeric, is a non-competitive selective PhK inhibitor (Reddy and Aggarwal 1994). It has also been shown to be a potent inhibitor of NF-kB activation (Singh and Aggarwal 1995) and may assist in blocking fibroblastic proliferation in thermal injuries. Phosphorylase kinase is activated within 5 mins of injury (Heng 2010, Heng and Heng 2014), and functions upstream of NF-kB (which is expressed 30 mins following injury), and much earlier than the appearance of activated fibroblasts (one week following injury) and myofibroblasts (2 weeks following injury). Accordingly, we hypothesized that blocking the activity of PhK is likely to have salutary effects in the treatment of burns and scalds by reducing the inflammatory response and the resulting tendency to hypertrophic scarring (Heng 2010, Heng 2013).

The following cases illustrate examples of patients with burns and scalds treated with topical curcumin gel that resulted in rapid healing and minimal or absent scarring.

Patient 1:

The patient is an 11 year old boy who sustained severe 2nd degree flash burns from pouring lighter fluid on warm barbeque coals. The heat from the ensuing fire singed his hair, eyelashes, forehead, ears, nose, cheeks and neck (Fig. 2, upper panel). He was treated with applications of curcumin gel applied hourly initially for several hours, and subsequently as frequently as possible during the first few days. When seen 5 days later, he was observed to be much improved, with re-epithelization of the raw areas over the forehead, ears, cheeks, nose and neck, and decreased edema over the affected areas, including the eyelids (Fig. 2, lower panel). At this time, pain was significantly decreased and reported to be absent most times. When seen 6 weeks later, the skin had healed completely, with some residual pigmentation over the most involved areas over the right cheek (Fig. 3). Follow up six months later revealed resolution of all pigmentary changes to resemble pre-injury skin (Fig. 4).

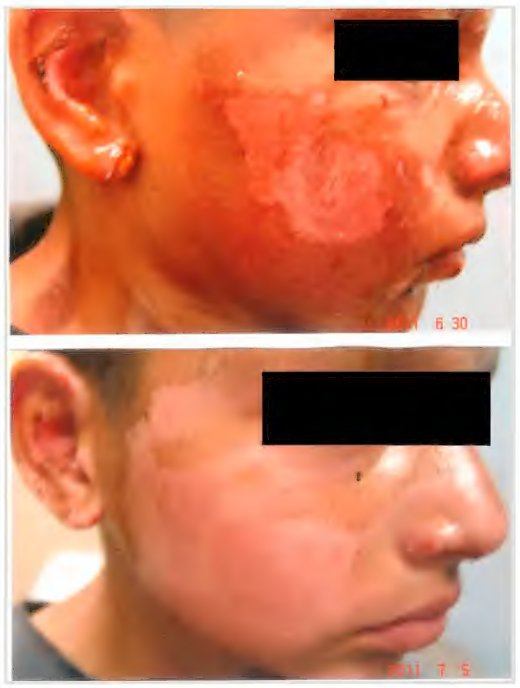

Figure 2.

Burns from Barbeque Fire:

- (upper panel): Second degree burns from a barbeque fire before treatment with curcumin gel.
- (lower panel): Rapid healing 5 days after curcumin gel applied hourly.

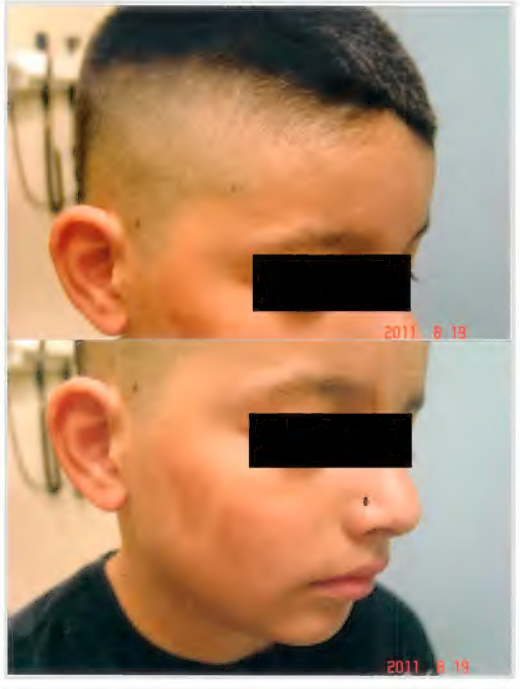

Figure 3.

Burns from Barbeque Fire:

- (upper panel): The singed hair from the burn were removed by a hair-cut. At 6 weeks after curcumin gel, the skin of the forehead, eyelids and ears were completely healed, with some residual pigmentation over the forehead.
- (lower panel): Residual hyperpigmentation right cheek 6 weeks following curcumin gel treatment.

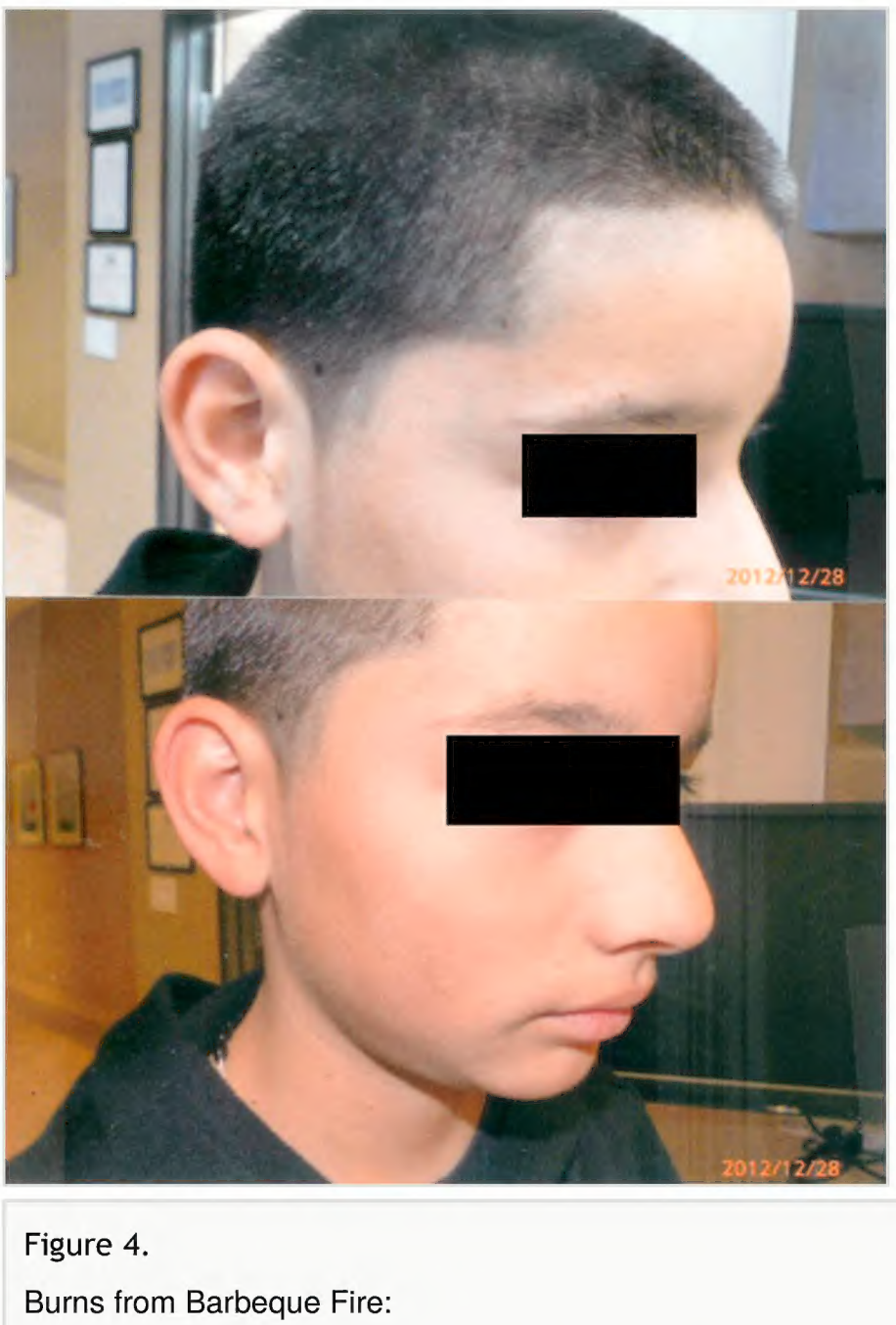

Figure 4.

Burns from Barbeque Fire:

- When seen several months after the burn injury, the patient was observed to be completely healed with no erythema, clinical scarring or pigmentary changes. There was also no neurological deficit.

Patient 2:

The patient was a 2-year old boy who sustained 2nd degree burns over the palms of both hands after falling into a camp-fire. He was seen at a number of emergency care centers, and treated with silvadene cream. When seen four days later, large blisters were seen over both palms (Fig. 5) and he was in a lot of pain. He was started on curcumin gel treatment (initial hourly applications) and was much improved when seen 24 hours later (Fig. 6), associated with marked decrease in pain. When seen 2 weeks later (Fig. 7), there was significant re-epithelialization with a few residual areas of incomplete re-epithelialization. The patient was not able to fully extend his fingers at this time (Fig. 7). When seen 2 months later, healing was complete, with no residual scarring or loss of function. The skin looked normal and the patient was able to extend his fingers fully (Figs 7, 8).

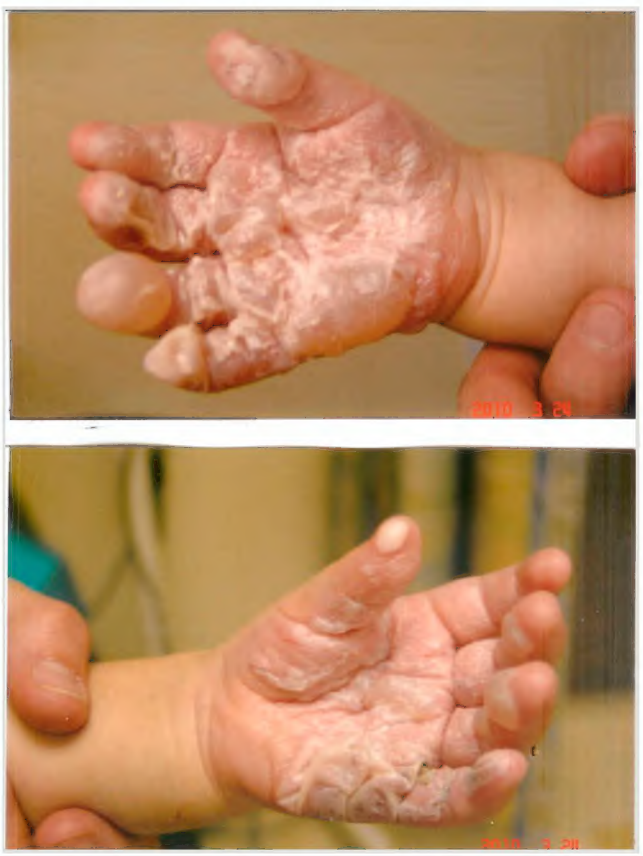

Figure 5.

Burns from a campfire see four days following injury, Note the presence of blisters suggesting at least second degree burns.

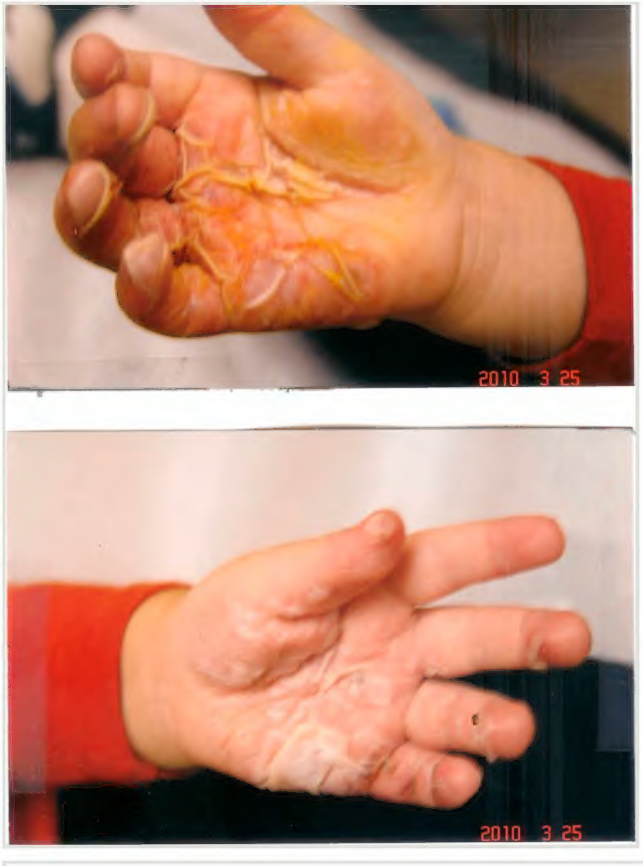

Figure 6.

Improvement one day later after hourly application of curcumin gel. Pain and blistering were much improved.

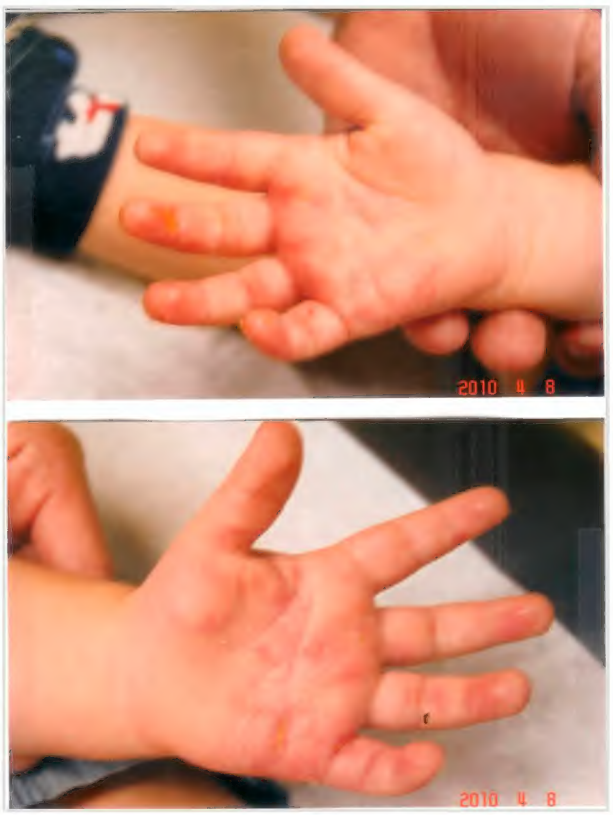

Figure 7.

Significant healing observed with curcumin gel (multiple applications daily) was observed after two weeks. Note rapid re-epithelialization of most of the skin of both palms. Also note inability of the patient to fully extend his fingers.

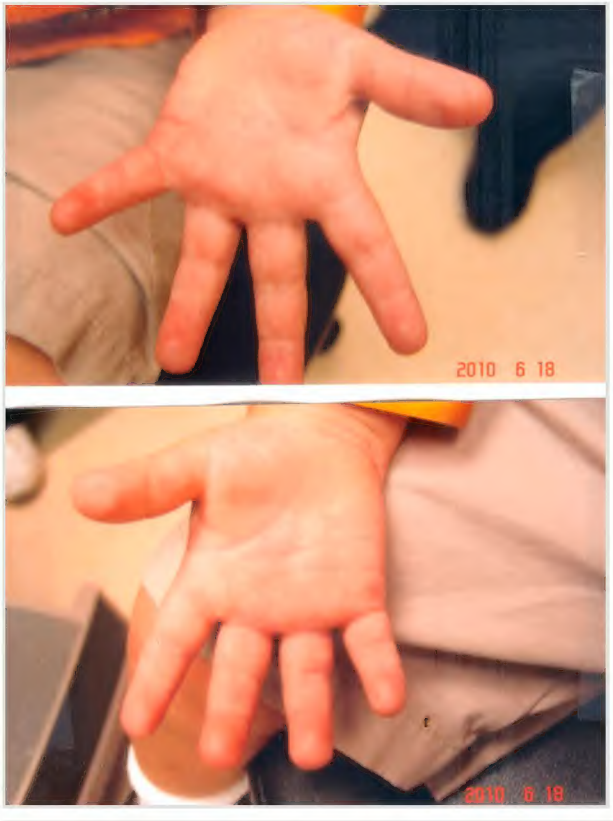

Figure 8.

Two months after curcumin gel treatment, there was complete healing of the skin of both palms, with no clinical scarring detected. The patient was able to fully extend all the fingers of both hands.

Patient 3:

The patient, a 35-year old female, sustained scalds to her left hand when she accidentally poured boiling water over the left hand. When seen one day later, early blister formation, suggestive of second degree injury, was observed both over the left palm and palmar aspect of all the fingers of her left hand (Fig. 9). Blister formation was also observed over the dorsum and fingers of the left hand (Fig. 9). She was in a lot of pain and was unable to fully extend the fingers of the injured hand (Fig. 9). She was treated with initial hourly applications of curcumin gel. When seen one day later (Fig. 10), she was much improved with aborted blister formation, decreased edema, and minimal pain. She was able to fully extend all the fingers of the injured hand (Fig. 10).

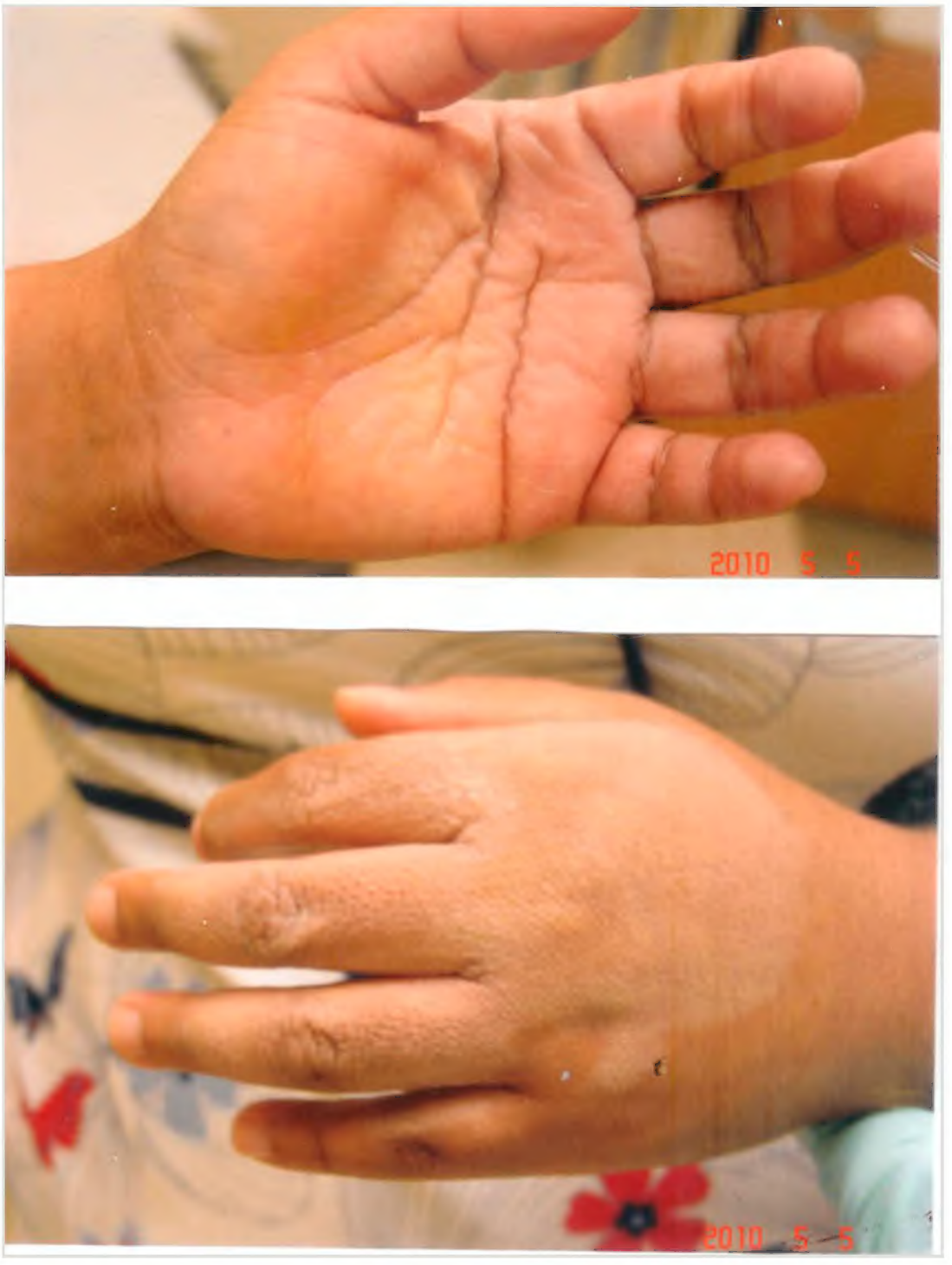

Figure 9.

Scald from boiling water seen one day after injury before curcumin gel. Note the presence of early blister formation associated with significant pain. Note also the inability of the patient to fully extend her fingers.

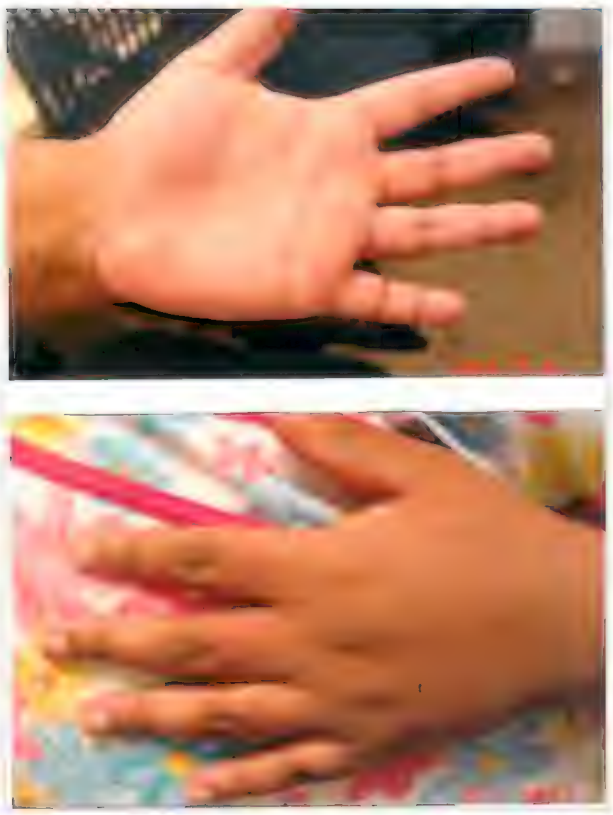

Figure 10.

Improvement 1 day after multiple applications of curcumin gel. Note aborted blister formation and greatly decreased pain. Note also the ability of the patient to fully extend her fingers without discomfort.

Potential Benefits of Curcumin in Thermal Injury - A Clinical Perspective

Wounds in adults, unlike fetal wounds, often heal with scarring (Martin 1997). Wounds resulting from burns and scalds are particularly predisposed to scar tissue formation, often resulting in unsightly, cicatricial scars which are usually hypertrophic, and occasionally leading to deformities (Hayakawa et al. 1979, Dunn et al. 1985, Robson et al. 1992). Current therapies for thermal burns include spray keratinocytes (Sood et al. 2015), autologous split thickness skin grafts (Sood et al. 2015), pedicle grafts (Gousheh et al. 2008), early excisions with grafting (Mohammadi et al. 2011) and reconstructive surgery (Gousheh et al. 2008, Kreymerman et al. 2011). Other less invasive therapies include compression therapy with pressure garments (Anzarut et al. 2009, Kim et al. 2015). Most of these therapeutic modalities have not resulted in clinically satisfactory normalization of function and scar-free healing.

In this review, we report the clinical outcome of several patients with burns and scalds treated with curcumin gel that resulted in minimal scarring or scar-free healing. We have previously reported similar beneficial outcomes with use of curcumin gel in post-surgical scars (Heng 2011). We hypothesize that the minimal scarring noted after these injuries may be due to inhibition of PhK activity by curcumin (Reddy and Aggarwal 1994, Heng 2011). Because PhK is released very early after injury (Yuan et al. 1993, Heng et al. 2000, Heng and Heng 2014), blocking PhK activity with curcumin results in significantly reduced

NF-kB-mediated inflammatory cell proliferation, and reduction of subsequent cytokine and growth factor-mediated fibroblastic proliferation and myofibroblastic transformation that follow one and two week later. Restated another way, the benefits of curcumin treatment after thermal injury are probably due to a reduction or inhibition of down-stream NF-kB-dependent events, including macrophage secretion of TGFβ1. Since TGFβ1 induces both fibroblastic proliferation and conversion of fibroblasts into myofibroblasts, the inhibition of the above mechanisms by curcumin may be responsible for the diminished scarring in thermal wounds in our patients treated with curcumin gel.

We noted that application of curcumin gel after burns and scalds is usually followed by rapid decrease in erythema, blistering, swelling and pain. Improvement in these clinical symptoms and findings strongly suggests that cytokine activity, in particular TNFα, is reduced in curcumin-treated wounds. TNFα is an inflammatory cytokine produced by many activated cells, and especially inflammatory cells such as T lymphocytes and macrophages. TNFα levels have been shown to be significantly increased in burns and other types of injury. By blocking PhK activity (Reddy and Aggarwal 1994), curcumin decreases NF-kB activity (Singh and Aggarwal 1995), and NF-kB-dependent cytokine (TNFα) secretion by inflammatory cells. Since NF-kB is also stimulated by TNFα (Ozes et al. 1999, Palkowitsch et al. 2008), thus providing a positive feed-back loop, suppression of NF-kB activity by curcumin gel protects against the deleterious effects of this cytokine after thermal injury. The rapidity of healing of burns and scalds with application of curcumin gel is particularly noteworthy from a clinical perspective and may involve several mechanisms. Besides that mentioned above with regard to its effects on cytokines, another reason for the salutary effects of curcumin may be through removal of damaged cells by the process of curcumin-induced apoptosis (Anto et al. 2002, Wang et al. 2009). The removal of damaged or dead cells may provide the space for replacement by new healthy cells – it could be speculated that it may be faster to grow new cells than to heal damaged ones. Yet another possible mechanism with curcumin use is a reduction in the incidence of bacterial colonization of the wound as result of more rapid healing, thus preventing secondary bacterial antigenic and lipopolysaccharide induced worsening of the wound (Gromkowski et al. 1990).

Conflicts of interest

Dr. Heng has shares in Omnicure Inc., a company that manufactures and markets topical curcumin gel.

References

- Abdou AG, Maraee AH, Al-Bara AM, Diab WM (2011) Immunohistochemical Expression of TGF-β1 in Keloids and Hypertrophic Scars. The American Journal of Dermatopathology 33 (1): 84-91. https://doi.org/10.1097/dad.0b013e3181d0c3ad

- Adzick N, Harrison M, Glick P, Beckstead J, Villa R, Scheuenstuhl H, Goodson W (1985) Comparison of fetal, newborn, and adult wound healing by histologic, enzyme-histochemical, and hydroxyproline determinations. Journal of Pediatric Surgery 20 (4): 315-319. https://doi.org/10.1016/s0022-3468(85)80210-4
- Anto R, Mukhopadhyay A, Denning K (2002) Curcumin (diferuloylmethane) induces apoptosis through activation of caspase-8, BID cleavage and cytochrome c release: its suppression by ectopic expression of Bcl-2 and Bcl-xl. Carcinogenesis 23 (1): 143-150. https://doi.org/10.1093/carcin/23.1.143
- Anzarut A, Olson J, Singh P, Rowe B, Tredget E (2009) The effectiveness of pressure garment therapy for the prevention of abnormal scarring after burn injury: a meta-analysis. Journal of plastic, reconstructive & aesthetic surgery: JPRAS 62 (1): 77-84. https://doi.org/10.1016/j.bjps.2007.10.052
- Armstrong J, Ferguson M (1995) Ontogeny of the Skin and the Transition from Scar-Free to Scarring Phenotype during Wound Healing in the Pouch Young of a Marsupial, Monodelphis domestica. Developmental Biology 169 (1): 242-260. https://doi.org/10.1006/dbio.1995.1141
- Bethea J, Castro M, Keane R, Lee T, Dietrich W, Yezierski R (1998) Traumatic spinal cord injury induces nuclear factor-kappaB activation. The Journal of neuroscience : the official journal of the Society for Neuroscience 18 (9): 3251-60.
- Clark R, Folkvord J, Hart C, Murray M, McPherson J (1989) Platelet isoforms of platelet-derived growth factor stimulate fibroblasts to contract collagen matrices. Journal of Clinical Investigation 84 (3): 1036-1040. https://doi.org/10.1172/jci114227
- Desmouliere A, Gabbiani G (1996) The Molecular and Cellular Biology of Wound Repair. Plenum Press, New York, 391-423 pp.
- Desmouliere A, Geinoz A, Gabbiani F (1993) Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. The Journal of Cell Biology 122 (1): 103-111. https://doi.org/10.1083/jcb.122.1.103
- Dunkin C, Pleat J, Gillespie P, Tyler M, Roberts A, McGrouther D (2007) Scarring Occurs at a Critical Depth of Skin Injury: Precise Measurement in a Graduated Dermal Scratch in Human Volunteers. Plastic and Reconstructive Surgery 119 (6): 1722-1732. https://doi.org/10.1097/01.prs.0000258829.07399.f0
- Dunn M, Silver F, Swann D (1985) Mechanical Analysis of Hypertrophic Scar Tissue: Structural Basis for Apparent Increased Rigidity. Journal of Investigative Dermatology 84 (1): 9-13. https://doi.org/10.1111/1523-1747.ep12274528
- Estes J, Vande Berg J, Adzick N, MacGillivray T, Desmoulière A, Gabbiani G (1994) Phenotypic and functional features of myofibroblasts in sheep fetal wounds. Differentiation 56 (3): 173-181. https://doi.org/10.1046/j.1432-0436.1994.5630173.x
- Ferguson MWJ, O'Kane S (2004) Scar-free healing: from embryonic mechanisms to adult therapeutic intervention. Philosophical Transactions of the Royal Society B: Biological Sciences 359 (1445): 839-850. https://doi.org/10.1098/rstb.2004.1475
- Gousheh J, Arasteh E, Mafi P (2008) Super-thin abdominal skin pedicle flap for the reconstruction of hypertrophic and contracted dorsal hand burn scars. Burns 34 (3): 400-405. https://doi.org/10.1016/j.burns.2007.03.025
- Grinnell F (1994) Fibroblasts, myofibroblasts, and wound contraction. The Journal of Cell Biology 124 (4): 401-404. https://doi.org/10.1083/jcb.124.4.401

- Gromkowski S, Mama K, Yagi J, Sen R, Rath S (1990) Double-stranded RNA and bacterial lipopolysaccharide enhance sensitivit to TNF-α -mediated cell death. International Immunology 2 (9): 903-908. https://doi.org/10.1093/intimm/2.9.903
- Hayakawa T, Hashimoto Y, Myokei Y, Aoyama H, Izawa Y (1979) Changes in type of collagen during the development of human post-burn hypertrophic scars. Clinica Chimica Acta 93 (1): 119-125. https://doi.org/10.1016/0009-8981(79)90252-3
- Heng M (2010) Curcumin targeted signaling pathways: basis for anti-photoaging and anti-carcinogenic therapy. International Journal of Dermatology 49 (6): 608-622. https://doi.org/10.1111/j.1365-4632.2010.04468.x
- Heng M (2013) Signaling pathways targeted by curcumin in acute and chronic injury: burns and photo-damaged skin. International journal of dermatology 52 (5): 531-43. https://doi.org/10.1111/j.1365-4632.2012.05703.x
- Heng M, Heng M (2014) Early events after skin injury: Observations on changes in Langerhans cells and Birbeck granules with role of phosphorylase kinase in the injury pathway. International Journal of Clinical and Experimental Physiology 1 (4): 283. https://doi.org/10.4103/2348-8093.149759
- Heng M, Song M, Harker J, Heng M (2000) Drug-induced suppression of phosphorylase kinase activity correlates with resolution of psoriasis as assessed by clinical, histological and immunohistochemical parameters. British Journal of Dermatology 143 (5): 937-949. https://doi.org/10.1046/j.1365-2133.2000.03767.x
- Heng MY (2011) Wound healing in adult skin: aiming for perfect regeneration. International Journal of Dermatology 50 (9): 1058-1066. https://doi.org/10.1111/j.1365-4632.2011.04940.x
- Karin M, Ben-Neriah Y (2000) Phosphorylation Meets Ubiquitination: The Control of NF-κB Activity. Annual Review of Immunology 18 (1): 621-663. https://doi.org/10.1146/annurev.immunol.18.1.621
- Kim J, Willard J, Supp D, Roy S, Gordillo G, Sen C, Powell H (2015) Burn Scar Biomechanics after Pressure Garment Therapy. Plastic and Reconstructive Surgery 136 (3): 572-581. https://doi.org/10.1097/prs.0000000000001507
- Kreymerman P, Andres L, Lucas H, Silverman A, Smith A (2011) Reconstruction of the Burned Hand. Plastic and Reconstructive Surgery 127 (2): 752-759. https://doi.org/10.1097/prs.0b013e3181fed7c1
- Lee T, Chin G, Kim W, Chau D, Gittes G, Longaker M (1999) Expression of Transforming Growth Factor Beta 1, 2, and 3 Proteins in Keloids. Annals of Plastic Surgery 43 (2): 179-184. https://doi.org/10.1097/00000637-199902000-00013
- Leibovich S, Ross R (1975) The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. The American journal of pathology 78 (1): 71-100.
- Martin P (1997) Wound Healing--Aiming for Perfect Skin Regeneration. Science 276 (5309): 75-81. https://doi.org/10.1126/science.276.5309.75
- Martin P, Dickson M, Millan F, Akhurst R (1993) Rapid induction and clearance of TGFβ1 is an early response to wounding in the mouse embryo. Developmental Genetics 14 (3): 225-238. https://doi.org/10.1002/dvg.1020140309
- Mohammadi A, Bakhshaeekia A, Marzban S, Abbasi S, Ashraf A, Mohammadi M, Toulide-ie H, Tavakkolian A (2011) Early excision and skin grafting versus delayed skin grafting in deep hand burns (a randomised clinical controlled trial). Burns 37 (1): 36-41. https://doi.org/10.1016/j.burns.2010.02.005

- Montesano R, Orci L (1988) Transforming growth factor beta stimulates collagen-matrix contraction by fibroblasts: implications for wound healing. Proceedings of the National Academy of Sciences 85 (13): 4894-4897. https://doi.org/10.1073/pnas.85.13.4894
- Ozes O, Mayo L, Gustin J, Pfeffer S, Pfeffer L, Donner D (1999) NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. Nature 401 (6748): 82-5. https://doi.org/10.1038/43466
- Palkowitsch L, Leidner J, Ghosh S, Marienfeld R (2008) Phosphorylation of Serine 68 in the I B Kinase (IKK)-binding Domain of NEMO Interferes with the Structure of the IKK Complex and Tumor Necrosis Factor - induced NF - B Activity. Journal of Biological Chemistry 283 (1): 76-86. https://doi.org/10.1074/jbc.m708856200
- Reddy S, Aggarwal B (1994) Curcumin is a non-competitive and selective inhibitor of phosphorylase kinase. FEBS Letters 341 (1): 19-22. https://doi.org/10.1016/0014-5793(94)80232-7
- Robson M, Barnett R, Leitch I, Hayward P (1992) Prevention and treatment of postburn scars and contracture. World Journal of Surgery 16 (1): 87-96. https://doi.org/10.1007/bf02067119
- Singh S, Aggarwal B (1995) Activation of Transcription Factor NF- B Is Suppressed by Curcumin (Diferuloylmethane). Journal of Biological Chemistry 270 (42): 24995-25000. https://doi.org/10.1074/jbc.270.42.24995
- Sood R, Roggy D, Zieger M, Nazim M, Hartman B, Gibbs J (2015) A comparative study of spray keratinocytes and autologous meshed split-thickness skin graft in the treatment of acute burn injuries. Wounds: a compendium of clinical research and practice 27 (2): 31-40.
- Springer T (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: The multistep paradigm. Cell 76 (2): 301-314. https://doi.org/10.1016/0092-8674(94)90337-9
- Takada Y, Singh S, Aggarwal B (2004) Identification of a p65 Peptide That Selectively Inhibits NF- B Activation Induced by Various Inflammatory Stimuli and Its Role in Down-regulation of NF- B-mediated Gene Expression and Up-regulation of Apoptosis. Journal of Biological Chemistry 279 (15): 15096-15104. https://doi.org/10.1074/jbc.m311192200
- Tredget E, Yang L, Delehanty M, Shankowsky H, Scott P (2006) Polarized Th2 Cytokine Production in Patients with Hypertrophic Scar Following Thermal Injury. Journal of Interferon & Cytokine Research 26 (3): 179-189. https://doi.org/10.1089/jir.2006.26.179
- Verma I, Stevenson J, Schwarz E, Antwerp D, Miyamoto S (1995) Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. Genes & Development 9 (22): 2723-2735. https://doi.org/10.1101/gad.9.22.2723
- Wang J, Qi L, Zheng S, Wu T (2009) Curcumin induces apoptosis through the mitochondria-mediated apoptotic pathway in HT-29 cells. Journal of Zhejiang University SCIENCE B 10 (2): 93-102. https://doi.org/10.1631/jzus.b0820238
- Wang J, Dodd C, Shankowsky H, Scott P, Tredget E (2008) Deep dermal fibroblasts contribute to hypertrophic scarring. Laboratory Investigation 88 (12): 1278-1290. https://doi.org/10.1038/labinvest.2008.101
- Whitby D, Ferguson M (1991) Immunohistochemical localization of growth factors in fetal wound healing. Developmental Biology 147 (1): 207-215. https://doi.org/10.1016/s0012-1606(05)80018-1
- Yang F, Yamashita J, Tang E, Wang H, Guan K, Wang C (2004) The Zinc Finger Mutation C417R of I- B Kinase Impairs Lipopolysaccharide- and TNF-Mediated NF- B

Activation through Inhibiting Phosphorylation of the I- B Kinase Activation Loop. The Journal of Immunology 172 (4): 2446-2452. https://doi.org/10.4049/jimmunol.172.4.2446

- Yuan C, Huang C, Graves D (1993) Phosphorylase kinase, a metal ion-dependent dual specificity kinase. The Journal of biological chemistry 268 (24): 17683-6.